Bioorganic & Medicinal Chemistry Letters
journal homepage: www.elsevier.com

Selective anti-malarial minor groove binders

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ARTICLE INFO

Article history:
Received
Revised
Accepted
Available online

Keywords:
DNA minor groove binder
Plasmodium falciparum
Antimalarial drug
Keyword_4
Keyword_5

ABSTRACT

A set of 31 DNA minor groove binders (MGBs) with diverse structural features relating to both physical chemical properties and DNA binding sequence preference has been evaluated as potential drugs to treat *Plasmodium falciparum* infections using a chloroquine sensitive strain (3D7) and a chloroquine resistant strain (Dd2) in comparison with human embryonic kidney (HEK) cells as an indicator of mammalian cell toxicity. MGBs with an alkene link between the two N-terminal building blocks were demonstrated to be most active with IC_{50} values in the range 30 – 500 nM and therapeutic ratios in the range 10 - > 500. Many active compounds contained a *C*-alkylthiazole building block. Active compounds with $logD_{7.4}$ values of approximately 3 or 7 were identified. Importantly the MGBs tested were essentially equally effective against both chloroquine sensitive and resistant strains. The results show that suitably designed MGBs have the potential for development into clinical candidates for antimalarial drugs effective against resistant strains of *Plasmodia*.

Global efforts in the control and prevention of malaria from 2000 to 2015 have dramatically reduced both the incidence (37%) and mortality (60%) rate due to malaria infections; however, the threat of parasite resistance is still threatening and could undermine such achievements. Three of the five Plasmodium species which are known to infect humans (*P. falciparum*, *P. vivax* and *P. malariae*) have all demonstrated resistance to common use antimalarials.[1] With resistance to Artemisnin monotherapy and also combination therapy (ACT) reported as a delayed clearance of infection with standard dosing regimens, there is a need for new antimalarial compounds.[2] Those with alternative modes of action are of most value as cross-resistance generated to all compounds within the same chemical class or mode of action is a common phenomenon.[1] Although work towards this goal is progressing, such as with DDD107498, there is still need to maintain a pipeline of potential novel therapeutics should resistance emerge.[3] A number of screening campaigns have identified new drug candidates which appear to be clustering to a small number of targets, for example *Pf*ATP4,[4] PI4K[5] and *Pf*DHODH[6]. The identification of compounds with alternative targets or modes of action is an obvious direction to take in order to minimize the threat of cross-resistance.

Minor groove binders (MGBs) are class of compound that bind to the minor groove of DNA and have found use as both human and animal antimicrobial therapies.[7-10] Of particular note is a class of MGB that is based on arylamidines such as berenil, pentamidine and DB75 (Figure 1), that have significant activity against many parasitic microorganisms.[8, 11] Pentamidine has been used clinically for over 60 years to treat many infectious diseases in humans, particularly human African trypanosomiasis; however, it has issues with adverse side effects and poor oral availability.[12] The arylamidines were used as the basis of a synthesis campaign by Boykin and coworkers which lead to the discovery of DB75, furamidine (Figure 1), a highly active antitrypanosomal drug in animal studies, and an orally available prodrug of it, DB289, pafuramidine (Figure 1).[13, 14] Possible renal toxicity in the later clinical trials lead to the development DB289 being paused; however, this near clinical success serves to highlight the potential of MGBs as antiparasitic therapies.[11]

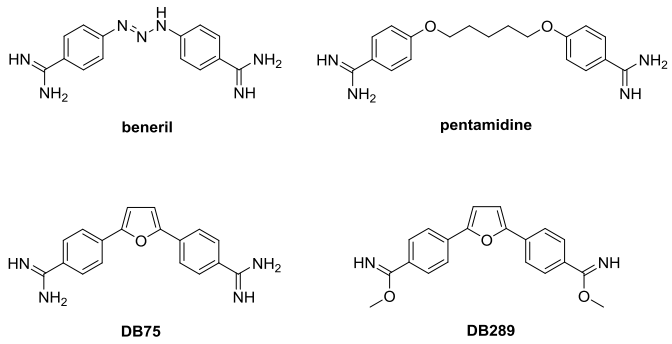

Figure 1. Examples of arylamidine minor groove binders.

Strathclyde minor groove binders (S-MGBs) make up a family of DNA-binding compounds loosely built upon the structure of

distamycin as a template but including a very wide range of structural components so that an extensive coverage of structural and property space can be obtained within the same DNA- binding template (Figure 2).[15,16] The principal variables are the head groups and their linkage to the rest of the molecule (amidine, amide, or alkene), the heterocyclic building blocks, the alkyl substituents on the heterocyclic building blocks, and the basicity of the C-terminal tail group. By manipulating these variables potent antibacterial compounds have been discovered, one of which has completed phase 1 clinical trials,[17] and other compounds have been shown to be active against *Trypanosoma* both in cell-based studies and in mouse models of disease and leishmaniasis (unpublished results).[18, 19] A potential benefit of targeting DNA is that it is to be expected that several binding sites will be occupied by S-MGBs leading to multiple mechanisms of action, which in turn could mitigate risks of rapid development of resistance. A counter balancing risk is that the ubiquity of DNA makes species selectivity critical. Studies of S-MGBs have shown that selectivity can be obtained between different infectious agents and mammalian cells with sufficient therapeutic ratios to indicate that candidate drugs can be obtained.[15, 16, 20] This paper describes the evaluation of a subset of S-MGBs covering the three head group linking types together with other variations in the context of antimalarial drug discovery comparing the activity in a chloroquine sensitive (3D7) and resistant (Dd2) strain of *Plasmodium falciparum.*

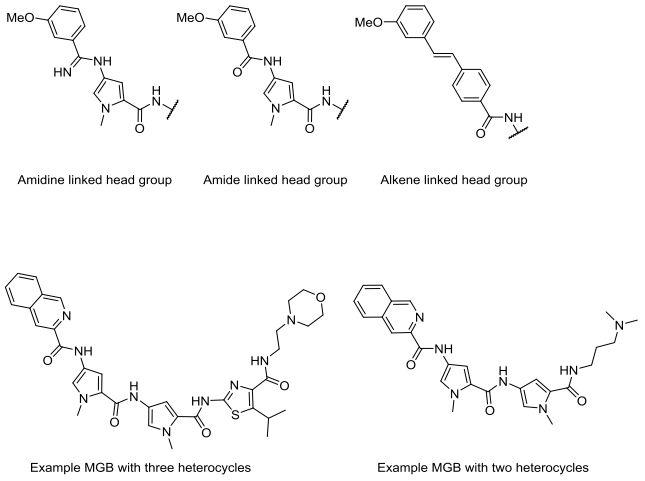

Figure 2. Examples of structures within S-MGBs. Using the shorthand structure code in the table, the head group fragments are 3-MeOC$_6$H$_4$(am)-PyMe-; 3-MeOC$_6$H$_4$-PyMe-; 3-MeOC$_6$H$_4$=C$_6$H-. The compound examples are 3-Isoquin-PyMe-PyMe-PyMe-MorphE and 3-Isoquin-PyMe-PyMe-DMAP.

The syntheses of the compounds evaluated have been described previously and all samples assayed were greater than 98% pure by HPLC and ^{1}H NMR.[15, 16] As shown in Table 1, the structures of the compounds included the three head group types (figure 1) with a range of polar and non-polar substituents, *N*-alkyl pyrroles, a standard feature of minor groove binders, *C*-alkyl thiazoles, a specific feature of S-MGBs, and a basic tertiary amine (dimethylamino) or weakly basic amine (morpholino) tail group. The *C*-alkyl thiazole is significant in that it promotes binding at GC sites in DNA whereas *N*-alkyl pyrroles bind preferentially at AT sites.[21] Different combinations of these components allow for a wide range of DNA-binding and physicochemical properties to be covered in a small number of compounds. For example, the clogD$_{7.4}$ values for the set range between -3.28 and 6.97 (Table 1).[19]

Compound inhibitory activity was determined in IC$_{50}$ dose response format against *Pf*3D7 (chloroquine sensitive) and *Pf*Dd2 (chloroquine resistant) stains using a previously described high content imaging assay.[22] In addition, compounds were simultaneously tested for general cytotoxicity against HEK293 cells using an AlamarBlue based assay which measures cell metabolic activity. All compounds were tested in duplicate point in 22 doses in two separate experiments.

The data shown in Table 1 illustrate both significantly active and inactive compounds (Structures shown in ESI). No significant activity was observed in compounds without an aromatic head group (**2**, **8**). Encouraging activity and selectivity were found almost entirely within the alkene-linked subset of compounds; the amidine-linked compounds were all at best weakly active and only one amide-linked compound had significant activity (**12**). Interestingly, this compound contains a *C*-alkylthiazole with a branched alkyl chain (*i*-propyl), a structural feature that seems to promote antimalarial activity. Overall, five of the most active compounds, all alkene linked, contained a *C*-alkylthiazole (**18-21**, **22**, **23**, **25**). Even in the weakly active amidine series, the *C*-alkylthiazole noticeably increased the activity (compare **3** and **5**). Importantly, approximately equal activity was observed between the resistant and sensitive strain, there being no statistically significant difference between the two data sets (two-tailed sign test, p = 1.000).

The antiparasitic and antibacterial activity of S-MGBs can be broadly considered in two principal dimensions, namely binding to DNA and access to cells. The latter has been found to be particularly important in the differences of behaviour of S-MGBs between Gram-positive and Gram-negative bacteria; antibacterial activity is strongly shown against the former but not the latter and there is good evidence that for Gram-negative bacteria, efflux pumps prevent the S-MGBs from reaching the target DNA (unpublished results). Interaction of S-MGBs with efflux pumps appears to be related to their physicochemical characteristics. These properties could also be relevant in antimalarial activity. Taking logD$_{7.4}$ as a measure of lipophilicity, these data suggest that a minimum value of about 2.9 is necessary for modest activity (IC$_{50}$ < 2 μM) against the two strains of *P. falciparum* tested.

Using these data as a guide it is possible to suggest that the most polar compounds such as the amidines (**1**, **2**) are unable to reach target DNA in plasmodia cells; this effect is evidently mitigated by the presence of the *C*-alkyl thiazole (**5**, **7**) which leads to significantly higher logD$_{7.4}$ values and activity less that 2 μM. Significant sub-micromolar activity, however, is restricted to the alkene series (**18-21**, **22**, **23**, **25**, **26**) and one amide (**12**); in the alkene series, the logD$_{7.4}$ values span a wide range (4.14 – 7.67) indicating that this parameter alone cannot account for the activity observed (Figure 3). Equally, it appears that toxicity as measured in HEK cells is not directly related to lipophilicity, which is significant for potential optimization of the leads.

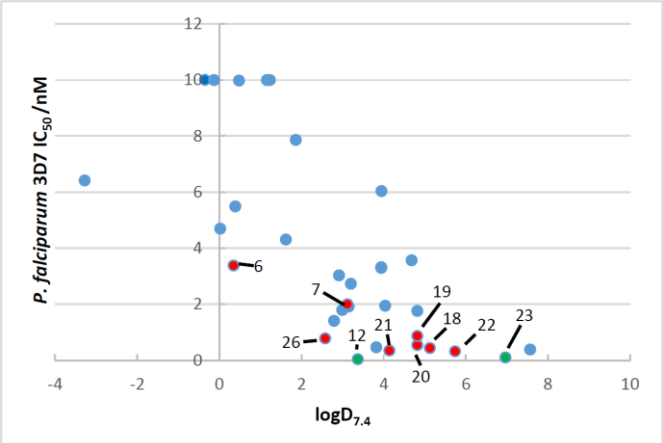

Figure 3. The relationship between activity and lipophilicity. The two most active compounds are shown in green. Those with measurable toxicity against HEK cells are shown in red.

As noted above, the presence of the *C*-alkyl thiazole appears to be significant. It is also plausible that *Plasmodia* could have a GC base pair at the DNA binding site of the S-MGB, unlike the antibacterial compounds, which are predominantly AT readers; this is notable bearing in mind the low GC content in the *P. falciparum* genome.[23] It is also notable that none of the compounds in this set showed high antibacterial activity, further emphasizing that selectivity of action is possible with S-MGBs.

Compound	% Inhibition or IC_{50} *P. falciparum* 3D7 (nM)	% Inhibition or IC_{50} *P. falciparum* Dd2 (nM)	% Inhibition or IC_{50} HEK cells (na = no effect @ 20 μM)	Selectivity HEK/3D7	$logD_{7.4}$
Amidines					
1	90% @ 20 μM	63% @ 20 μM	na		-0.35
2	6422	6457	na		-3.28
3	2731	2970	na		3.2
4	9980	100% @ 20 μM	na		0.48
5	na	35% @ 20 μM	na		1.23
6	3384	2773	40% @ 10 μM		0.35
7	1997	1507	76% @ 20 μM		3.12
Amides					
8	98% @ 20 μM	25% @ 20 μM	na		-0.13
9	1800	3072	na		2.99
10	3035	3617	na		2.91
11	75% @ 20 μM	65% @ 20 μM	na		1.16
12	469	636	na	>42	3.82
13	7860	5990	na		1.86
14	4312	3267	na		1.62
15	4701	4348	na		0.02
16	1409	1999	na		2.79
17	5491	4479	na		0.39
Alkenes					
18	437	288	7.65 μM	15.8	5.13
19	544	1132	86% @ 20 μM	> 36	4.82
20	868	1148	5.8*	6.7	4.82
21	350	339	60% @ 20 μM	>14	4.14
22	322	289	54% @ 20 μM	> 60	5.74
23	390	588	na	> 50	7.57
24	3570	3691	na		4.68
25	103	241	na	> 194	6.97
26	780	923	63% @ 20 μM	> 26	2.58
27	39	38	na	> 514	3.37
28	3297	3341	na		3.94
29	3322	2747	na		3.94
30	6037	5144	na		3.95
31	1926	2302	na		3.15
32	1947	2986	na		4.04
33	1766	1899	na		4.82
Controls					
Puromycin	60	62	0.350	6	-
Pyrimethamine	8.45	>120uM	8.07	955	-
Chloroquine	11.35	177	>120	>10000	-
Artemisinin	7.75	9	>120	>10000	-

Table 1. Biological compounds of S-MGBs evaluated in this study. LogD7.4 predicted using the software MarvinSketch (Version 15.6.29.0, ChemAxon, http://www.chemaxon.com).

The therapeutic ratio between infected and non-infected cells is also an important criterion in assessing the potential of compounds as leads for drug discovery. In this study, the viability of HEK cells was used as the measure of potential cytotoxicity to a human host. Unsurprisingly compounds that contained a known toxophore (aromatic nitro groups, **21**, **22**) showed toxicity when HEK cells were treated with 20 μM S-MGB. Even these compounds, however, had a reasonable therapeutic ratio (>14, >60) and other compounds (**12**, **14**, **18**, **19**, **21**) were comparable in this respect. The equivalent activity witnessed for both the *Plasmodium falciparum* strains demonstrates no common resistance mechanism shared by these compounds.

One compound (**27**), with the benzoxazole containing head group stands outside this analysis with a $logD_{7.4}$ appropriate for further development (3.37) and high antiparasitic activity (~ 40 nM) which, in the absence of a *C*-alkylthiazole, imply a significant and unexpected role for the head group. This result is also surprising because S-MGBs (**26**, **30 - 32**) with other strongly hydrogen bonding aromatic head groups and otherwise identical structures in the rest of the S-MGB were greatly inferior even if the $logD_{7.4}$ value was similar to that of **27** as is the case with the oxadiazole, **31**.

There are many structural variables to manipulate in the S-MGB structure. This study shows that active and selective compounds can be found with both moderate and high $logD_{7.4}$ values (**25** and **27**, Figure 4). It would normally be expected that the compound with the lower $logD_{7.4}$ has the greater potential for development into a drug and the benzoxazole head group in **27** has scope for incorporation into compounds containing the *C*-alkyl thiazoles, which were found to promote activity in many compounds. Perhaps the most important conclusion from this study is that S-MGBs are active against *P. falciparum* including a chloroquine-resistant strain and have a good potential therapeutic window (> 200 - > 500) for further development into lead optimization.

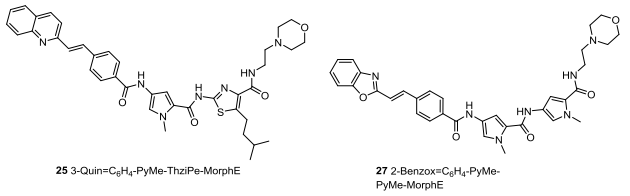

Figure 4. The two most active compounds (**25** and **27**) found in this study.

Acknowledgments

We thank Prof Michael Barrett, University of Glasgow, for facilitating the provision of compounds for assay in Australia.

The authors thank and acknowledge the Australian Red Cross Blood Bank for the provision of fresh red blood cells. This work was supported by the Australian Research Council (LP120200557 to VMA).

Supplementary Material

Supplementary data associated with this article, including full structural formulae, can be found, in the online version, at XXX

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